Effect of Plastic Packages on Benzo[*a*]pyrene Concentration in Sunflower Oil

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Abstract

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Commercially available sunflower oil and the same oil distilled additionally in a molecular evaporator (to remove naturally occurring compounds) was spiked with benzo[*a*]pyrene (BaP) at the levels of 37.1 and 38.6 μg/kg, respectively. Then, it was filled into polyethylene terephtalate (PET) and low density polyethylene (LDPE) receptacles of cylindrical shape, and BaP concentration was followed within 49 h. At the end of the experiments, BaP concentration in the non-distilled oil packed into PET decreased to 25.9 μg/kg, and BaP concentration in the distilled oil decreased to 34.6 μg/kg. The rate and the extent of BaP removal were evaluated comparing the diffusion and equilibrium coefficients. The results showed that PET is able to reduce BaP concentration in sunflower oil due to BaP sorption on the PET surface, but the rate and the extent of BaP removal are also affected by other compounds present in the oil. As found, LDPE is an inappropriate material for the BaP removal from sunflower and rapeseed oils, because BaP concentration in the oils remained at a constant level during the whole experiment.

Keywords: polycyclic aromatic hydrocarbons; benzo[*a*]pyrene; sunflower oil; polyethylene terephtalate; polyethylene

Polycyclic aromatic hydrocarbons (PAHs) belong to hazardous contaminants due to their known or suspected carcinogenicity and/or mutagenicity. In general, PAHs are formed by incomplete combustion of fossil fuels and other forms of organic matter. For this reason they are found in all parts of the environment, including foods (Tamakawa 2004). Moreover, PAHs are also formed in thermal processes during food production such as baking, grilling, roasting, frying, and smoking (Tamakawa *et al.* 1996; Chen 1997). With regard to the harmful effects of PAHs on living organisms, there are trends to enact maximum limits of these compounds in various foods to protect the consumers against harmful effects of these compounds. To simplify the difficulties associated with the great variability of PAH composition, BaP has been accepted, in general, as the indicator of the total PAH presence in foods regardless of the fact that BaP constitutes only 1–20% of the total carcinogenic PAHs (Andelman & Suess 1970). Relating to PAH legal limits, the situation in EU is changing now considerably due to the adoption of regulation 208/2005 limiting the BaP content to the level of 2 μg/kg in oils and fats intended for the direct human consumption or the use as an

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ingredient in foods (EC 2005a). This regulation has entered into power since 28th February 2005 and started to be applied as from 1st April 2005. Apart from this, EC has also adopted either directive 2005/10/EC laying down the sampling methods and the methods of analysis for the official control of BaP levels in foodstuffs (EC 2005b), or recommendation 2005/108/EC on further investigation into the levels of PAHs in certain foods as follows: benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene, BaP, chrysene, cyclopenta[*c, d*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,c*]anthracene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[*1,2,3-c,d*]pyrene, and 5-methylchrysene (EC 2005c). Fats and oils belong to the most important sources of PAH intake due to their extensive consumption as well as PAH concentrations which can sometimes reach high levels. For example, Moret *et al.* (1997) analysed 51 samples of olive oils and found the total PAH concentrations from 2.94 to 143.12 μg/kg. Hopia *et al.* (1986) found various PAHs in Finnish butters, margarines, and vegetable oils, and in some raw vegetable oil materials where total PAH concentrations in 25 samples varied from 0.17 μg/kg (corn oil) to 4600 μg/kg (crude coconut oil). It was concluded that the enormous PAH concentration in coconut oil could be caused by the direct drying of copra with smoke. Barranco *et al.* (2004) pointed out to the technological procedures of deodorisation and bleaching which are able to reduce considerably PAH concentrations in oils during the production. However, over-limit concentrations of BaP in Spanish olive oils was the reason to withdraw them from central European markets (Šimko *et al.* 2004). Polymers could play an important protective role with regard to the high affinity of PAH to some plastic materials (Šimko 2005). For example, PAH concentrations were effectively reduced in the liquid smoke flavour (by two orders) during 14 days of storage in LDPE flasks. As found, the rate-limiting step of the PAH sorption from a liquid into LDPE is the diffusion in liquid media (Šimko *et al.* 1994). During the interaction with LDPE, PAHs are primarily adsorbed on the LDPE surface with subsequent diffusion into the polymer bulk (Šimko *et al.* 1999). The most intense processes of PAHs removal from liquid media into LDPE take place within 24 h, where the polarity and viscosity of the liquid media also play an important role. During this period, the PAH concentration dropped in three various liquid systems by more than 50% in comparison to the initial concentration of 50 μg/kg of each compound tested (Chen & Chen 2005). The ability of PET to lower PAH concentrations in polar and non-polar liquid media has already been unambiguously proven (Šimko *et al.* 2004). However, the extent of the removal processes is strongly affected by the presence of other PAH compounds as well as other compounds such as vitamins, sterols, and waxes present in rapeseed oil (Šimko *et al.* 2005). With regard to the current knowledge mentioned above, the aim of this work was to study the possible effects of PET package on BaP concentration in sunflower oil, and of LDPE package in sunflower and rapeseed oils.

MATERIALS AND METHODS

Commercially available sunflower and rapeseed oils were purchased in a local market in Bratislava. The oils were packed in PET bottles of the volume of 4 l. Free fatty acids were removed during the production of the oils by chemical neutralisation.

A part of sunflower oil mentioned above was redistilled using molecular distillation equipment under lowered pressure of 10–20 Pa, and at the temperature of surface heating 200°C to remove naturally occurring compounds.

In the experiment, pre-bubbled PET receptacles of cylindrical shape with i.d. of 21.4 mm were used. The receptacles were provided by Palma-Tumys Ltd., Slovakia. The company uses them for oil and fruit syrup packaging after blowing to the volume of 2 litre.

LDPE cylindrical shape receptacles having i.d. of 32 mm were supplied by Cechvalab Ltd., Slovakia.

BaP of analytical grade was purchased from Supelco in the solid state. The solution for spiking was prepared by dissolving BaP in acetonitrile to the initial concentration of 500 mg/l.

Acetonitrile was of gradient grade (Merck, Germany), methanol and hexane (Slavus, Slovak Republic) were of analytical grade. The solvents were rectified immediately before use in a distillation apparatus.

Anhydrous Na_2SO_4 and alumina were purchased from Merck, Germany.

Experiment

First of all, the oils were analysed for the presence of BaP. Consequently, 100 g of oil was spiked with BaP solution in 2 l volume glass flask and the

solvent was allowed to evaporate spontaneously. To accelerate the evaporation, the oil was stirred intermittently. Then, roughly 900 g of the oil was added and the content of the flask was mixed thoroughly. At this stage, a sample of spiked oil was taken to determine the initial BaP concentration. Then, the PET and LDPE receptacles were filled with the spiked oil and placed into a polystyrene box to protect them from light and to keep then at a constant temperature. The samples for the analysis were taken after 1, 3, 5, 7, 11, 24, and 49 h. To maintain the same static conditions and sampling during the experiments, a new set of receptacles was taken for each analysis.

Sample preparation. The sample preparation was performed according to ISO 15302 as follows: 2 g of oil was weighed with the precision of 0.001 g into a 10 ml graduated flask, dissolved in hexane and diluted to the mark. Then, 22 g of alumina was transferred immediately to a chromatography column filled with hexane and anhydrous Na_2SO_4 was added to the top of the column to form a layer about 30 mm thick. Hexane was let to fall to the level of the top of Na_2SO_4 layer, and 2 ml of the graduated flask content was then applied onto the column. The column was eluted with hexane at a flow of about 1 ml/min, the first 20 ml of the eluate were discarded and then 60 ml of the eluate were collected in a 100 ml round-bottomed flask. The eluate was evaporated to about 0.5 ml and transferred into a vial. The evaporation continued under nitrogen until the residue was nearly dry. The round-bottomed flask was rinsed twice with about 1 ml of hexane and transferred quantitatively to a mini-vial where the evaporation continued under nitrogen. The evaporation was carried out to dryness, the residuum was then dissolved in methanol and analysed using HPLC.

HPLC analysis. The analyses were performed on the liquid chromatograph Agilent Technologies 1100 Series (Halbron, Germany) consisting of a quaternary pump, micro vacuum degasser, autosampler, and fluorescence detector, which operated at 300 nm excitation and 410 nm emission wavelengths. The separations were carried out at ambient temperature on LiChrolut column (25 cm × 0.4 cm i.d. packed with Lichrospher PAH, particle diameter 5 μm), when pre-column LiChroCart (4 cm × 0.4 cm) with the same particle diameter was also used. The flow rate of the mobile phase was 1 ml/min. For the determination, the gradient elution was used as follows: A – deionised water, B – acetonitrile. Gradient programme: from 0 to 2 min elution with 30% A and 70% B, then from 2 to 5 min with from 70% B to 100% B linearly, then the elution from 5 to 10 min isocratically, then from 10 to 15 min with from 100% B to 70% B linearly. The equilibration time between each run was 5 min. The samples were applied using an autosampler needle with 20 μl volume. All analyses were carried out in duplicates with an average relative standard deviation of 8.4%.

RESULTS AND DISCUSSION

At first, the experiment was carried out with commercially available sunflower oil to be filled in PET receptacles. The experimentally obtained dependences of BaP concentrations *vs*. time were used for the calculation of the diffusion coefficient *D* using a kinetic equation (1), which was derived for the diffusion of PAHs in non-stirred liquids placed into cylindrically shaped plastic bulks (Šimko *et al.* 2004):

$$c_t = c_\infty + (c_0 - c_\infty) \sum_{n=1}^{\infty} \frac{4}{a^2 \alpha_n^2} \exp\left[-D\alpha_n^2 t\right] \quad (1)$$

D was calculated by the non-linear least squares method by minimising the sum of squares of differences between the BaP concentrations measured experimentally and those calculated by equation (1). BaP equilibrium concentration between the oil and PET expresses the equilibrium coefficient β:

$$\beta = \frac{c_0 - c_\infty}{c_\infty} \quad (2)$$

where:

c_0 – initial BaP concentration in the oil

c_∞ – stands for the equilibrium BaP concentration in the oil in infinite time

The higher value of β coefficient corresponds to a greater decrease of BaP concentration in the liquid media. As follows from Figure 1, BaP concentration in the oil began to decrease immediately after filling the PET receptacles due to BaP sorption on the PET surface. The equilibrium concentration of 25.9 μg/kg was reached within about 24 h of the experiment which is the value already observed in rapeseed oil packed in PET (Šimko *et al.* 2005). As the value of the equilibrium coefficient β shows (Table 1), the elimination of BaP from the oil was quite efficient. Its extent is comparable with that one determined for rapeseed oil which confirms that the same removal processes took place also in

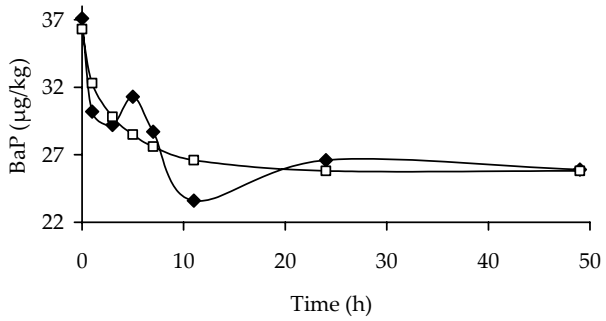

Figure 1. Changes in BaP concentration in sunflower oil stored in PET receptacles

◆ – experimentally obtained data (every point is average value of four BaP determinations with standard deviation = 1.2 µg/kg); □ – calculated data using kinetic equation (1)

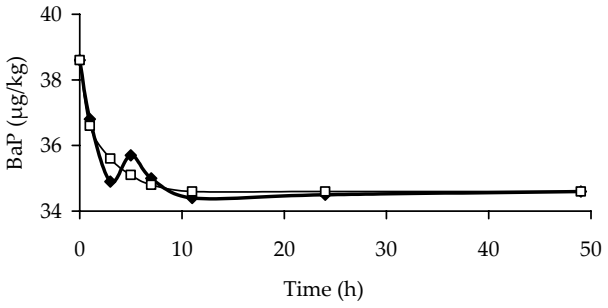

Figure 2. Changes in BaP concentration in additionally re-distilled sunflower oil stored in PET receptacles

◆ – experimentally obtained data (every point is average value of four BaP determinations with standard deviation = 0.9 µg/kg); □ – calculated data using kinetic equation (1)

the system PET-sunflower oil. However, the results obtained in the experiments with the re-distilled oil are very surprising (Figure 2). In general, the distillation (called also physical neutralisation) is used in oil industry as an alternative to the chemical neutralisation to remove free fatty acids from raw oils. Nonetheless, during the distillation other compounds are also removed (e.g. waxes, vitamins, sterols, etc.) which gives rise to almost a pure triacylglycerol mixture formation. The removal of these compounds from rapeseed oil increased the equilibrium coefficient as shown in Table 1 (Šimko *et al.* 2005). However, the distillation of sunflower oil brought about a considerable decrement of the equilibrium coefficient although the diffusion coefficient had an almost identical value. This fact was really unexpected because our aim was to remove the components competing with BaP for the adsorption centres on the PET surface. However, the decrease of the equilibrium coefficient observed could be explained by a matrix effect due to the different compositions of the vegetable oils. Oleic acid is a dominant fatty acid in rapeseed oil (62%) while linoleic acid is dominant in sunflower oil (63%). While oleic acid is mono-unsaturated, linoleic acid is di-unsaturated fatty acid which means that sunflower oil contains more double bonds, e.g. π-electron pairs, in comparison to rapeseed oil. PAHs are compounds rich in π-electrons localised in the condensed aromatic rings which invokes an idea of their interaction with π-electrons of linoleic acid. In non-distilled sunflower oil, π-electrons probably interact with naturally occurring compounds also rich in π-electrons, e.g. vitamin E. These compounds were removed by molecular distillation, and then the liberated π-electrons of linoleic acid could interact with π-electrons of BaP, which was revealed by the decreased value of the equilibrium coefficient. This assumption can be supported by the great similarity of the diffusion coefficient values for both sunflower oils (Table 1), which evokes that the main part of BaP interacted with π-electron pairs of linoleic acid, and that only a minor part of BaP interacted with PET. Overall, this "double behaviour" of BaP

Table 1. Parameters calculated from the experimentally measured values

	Diffusion coefficient D (cm^2/h)	Equilibrium coefficient β	Temperature during experiment (°C)
Sunflower oil	3.9×10^{-2}	0.407	23.2 ± 0.3
Re-distilled sunflower oil	6.6×10^{-2}	0.116	22.4 ± 0.3
Rapeseed oil*	2.5×10^{-2}	0.302	18.3 ± 0.4
Re-distilled rapeseed oil*	1.8×10^{-2}	0.371	20.4 ± 0.3

*values taken from Šimko *et al.* (2005)

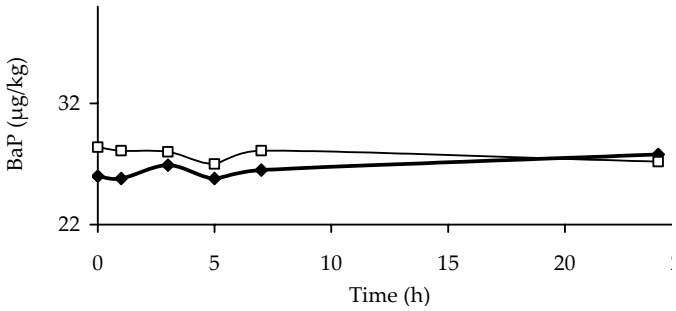

Figure 3. BaP concentration in sunflower (□) and rapeseed (◆) oils stored in PE receptacles (every point is average value of four BaP determinations with standard deviation = 0.3 µg/kg)

resulted in the lowered efficiency of the processes of BaP removal from distilled sunflower oil. Because the removal of BaP is limited by the surface adsorption and the diffusion of BaP into LDPE bulk had been already proven, LDPE was studied for BaP removal from the oil. However, BaP concentration in sunflower oil remained at a constant level, as shown in Figure 3, which was quite surprising. To assure this finding, the experiment was repeated with sunflower oil having been replaced with rapeseed oil. In spite of this change, the concentration remained at the constant level again, as shown in Figure 3. This observation is really surprising with regard to the previous findings because LDPE functioned effectively and it was able to decrease PAH concentration by one (Chen & Chen 2005) or two orders (Šimko *et al.* 1994) in liquid media. One explanation may reside in the fact that LDPE was used effectively for the removal of PAHs from polar or semi-polar liquid media. Oils, however, are either a non-polar matrix or contain double bonds able to interact via π-electron pairs with delocalised π-electrons of PAHs. As these interactions are probably stronger in comparison to the interactions of PAHs with LDPE (which is free of π-electron pairs), they are able to hinder the migration from PAH into LDPE and maintain the BaP concentration at a constant level. As can be seen, Figures 1 and 2, the dependences of BaP concentrations on time exhibit oscillations, especially at the beginning of the adsorption processes. These oscillations occur not only in both types of sunflower oil but they have already been observed also in rapeseed oil. Moreover, they were also observed in paraffin oil where the concentration was measured directly without the sample preparation procedures, just to exclude the possible experimental errors (Šimko *et al.* 2005). This indicates that the adsorption processes in the systems PET-vegetable oils are complicated or even combined with other processes unknown by this time.

CONCLUSIONS

The results and findings of this work lead to the following conclusions:

1. The BaP concentration in the sunflower oil filled into PET can decrease due to the interaction between BaP contained in the oil and PET.
2. The rate of BaP diffusion in the liquid phase and the extent of its adsorption onto PET depend on the presence of other compounds contained in the oil.
3. The removal of these compounds by distillation brings about a decrease in the extent of the BaP removal, probably due to the intensification of BaP and the oil matrix interactions through π-electrons.
4. From this point of view, the chemical neutralisation for free acids removal from sunflower oil should be preferred to the physical process of distillation.
5. LDPE is not an appropriate material for the elimination of PAHs from a non-polar matrix, e.g. sunflower and rapeseed oils.
6. The dependence of BaP concentrations on time exhibits oscillations; the reason for their existence is so far unknown.
7. This knowledge may be utilised in the industrial production of vegetable oils inserting an additional operation of PAH sorption on the surface of the PET particles just after the bleaching procedures to remove the residual PAHs, and in such way to protect human organism against the exposure to these carcinogenic compounds.

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